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14. ABSTRACT This project is a traineeship for the Primary Investigator's doctoral training and encompasses a training and research plan in breast cancer research. The PI will be mentored by three prominent researchers with expertise in multiple areas of breast cancer research: coordination and execution a highly-controlled breast cancer prevention feeding trial by Susan Raatz, PhD, R.D.; exposure to clinical oncology and clinical application of the results of the proposed research project by Douglas Yee, M.D.; and laboratory analysis of sex hormones and dietary prevention of breast cancer by Mindy Kurzer, PhD. The PI passed the oral preliminary exam in September 2007 and will complete all required course work by December of 2007. The purpose of the proposed dietary intervention trial is to determine the effects of type and amount of dietary fat on sex hormone metabolism, eicosanoid balance, and breast cancer risk in postmenopausal women. The study objectives are to: 1) evaluate the effects of total fat and omega-3 fatty acid intake on plasma and urinary sex hormone and urinary eicosanoid levels; 2) determine the relationships among plasma fatty acids, urinary prostaglandin E ₂ , plasma and urinary sex hormones, and plasma insulin, insulin-like growth factor, and insulin-like growth factor binding proteins. Plasma estradiol (E ₂), estrone (E ₁), estrone sulfate (E ₁ -S), testosterone (T), androstenedione (AS), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) were analyzed by radio-immunoassay for 10 participants. SHBG levels were significantly increased at 8 weeks with low fat high omega-3 diet (LFn3) compared to low fat diet (LF) (p < 0.05), and there was a trend for decreased DHEAS level at 8 weeks with LFn3 compared to LF (p < 0.15). A trend for increased E ₂ was observed with high fat diet (HF) compared to both LF and LFn3 at 8 weeks (p < 0.15). A trend for decreased E ₁ and FSH was observed from baseline to 8 weeks with LFn3 (p < 0.15). No statistically significant differences were observed between treatments for A, T, E ₁ -S, or DHEA. Preliminary results suggest that LFn3 alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women. Preliminary results suggest that the low-fat high omega-3 alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women.					
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Introduction

The training program funded by this proposal is an innovative combination of mentoring in execution of human clinical trials, training in nutritional sciences research and dietary prevention of breast cancer, and clinical mentoring by an oncologist. These aims support the PI's goal to become a clinical researcher in breast cancer prevention. The human feeding trial in this project, "Dietary Fat, Eicosanoids, and Breast Cancer Risk", aims to evaluate the effects of total fat intake and omega-3 fatty acids on breast cancer risk markers in postmenopausal women. This study will take place at the General Clinical Research Center (GCRC) at the University of Minnesota. Under the guidance of Susan Raatz, PhD, RD, the PI will recruit and screen subjects, manage data, and attend subject visits to the clinic. The PI will assist in the analysis of biological samples with trained technicians at the GCRC core laboratory and in the lab of Mindy Kurzer, PhD. The data resulting from this project will be the basis of the PI's PhD dissertation.

Body

This project is proceeding along the timeline outlined in the approved Statement of Work.

Task 1: The PI has been coordinating the proposed study over the past year by advertising for and screening subjects, managing data, ensuring proper study meal preparation, communicating with subjects and ensuring subject compliance.

Task 2: The PI has shadowed Douglas Yee, M.D., on clinical rounds and attended weekly discussion sessions with oncologists, radiologists, surgeons, and pharmacists to discuss case studies.

Task 3: The PI has assisted in the analysis of plasma sex hormones with radioimmunoassay kits (DSL, Austin TX) for 10 subjects. Fasting plasma samples from the baseline, 4 week and 8 week time points for each of the three diets (high fat diet, low fat diet, and low fat + omega-3 diet) for each participant were analyzed. Plasma samples were analyzed according to the "Materials and Methods, Serum collection and analysis" portion of Appendix 1, a paper from the lab of Dr. Mindy Kurzer, who is mentoring the PI on this part of the project. This was the first time samples were analyzed for this project, so there is no comparison to previous reports to be reported.

Appendix 2 includes the resulting preliminary data from the sex hormone analysis. The data table reports means \pm standard deviation for each hormone analyzed: estradiol (E_2), estrone (E_1), estrone sulfate (E_1 -S), testosterone (T), androstenedione (AS), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS). Data was analyzed by Minitab statistical software. Changes in each hormone concentration within treatment (comparing baseline to 8 week values) were tested using paired t-tests. Differences between each hormone concentration for the three treatments (comparing 8 week values between two different treatments) were compared using two-sample t-tests. SHBG levels were significantly increased at 8 weeks with the low fat + omega-3 diet compared to the low fat diet ($p < 0.05$), and there was a trend for decreased DHEAS level at 8 weeks with the low fat + omega-3 diet compared to the low fat diet ($p < 0.15$). A trend for increased E_2 was observed with the high fat diet compared to both the low fat and the low fat + omega-3 diet at 8 weeks ($p < 0.15$). A trend for decreased E_1 and FSH was observed from baseline to 8 weeks with the low fat + omega-3 diet ($p < 0.15$). No statistically significant differences were observed between treatments for A, T, E_1 -S, or DHEA. Preliminary results suggest that the low fat + omega-3 diet alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women. The full effects of the three diets on plasma sex hormone profile will be further elucidated as more subjects complete the study.

Urinary hormones have not yet been analyzed.

Tasks 4 and 5: Plasma fatty acids and urinary prostaglandin E_2 and thromboxane B_2 have not yet been analyzed.

Key Research/Training Accomplishments

- PI passed the oral preliminary exam to become a doctoral candidate in September, 2007.
- Preliminary data from plasma sex hormone analysis supports low fat, high omega-3 fatty acid diet in prevention of breast cancer by reducing hormone concentrations associated with increased breast cancer risk. See Appendix 2 data table for preliminary data results.

Reportable Outcomes

- Abstract (see Appendix 3) from The Conference on Bioactive Lipids in Cancer, Inflammation and Related Diseases, Montreal, Quebec, Sept 2007.

Conclusion

Recruitment and execution of the clinical trial portion of this project are proceeding smoothly. Analytes mentioned in the Statement of Work will be analyzed in batches as more subjects complete the study. The PI is proceeding towards the goal of attaining a PhD and receiving training to become a clinical researcher in the field of nutrition and breast cancer prevention.

Preliminary results of sex hormone analysis suggest that the low fat + omega-3 treatment alters sex hormones in a direction associated with reduced risk of breast cancer.

References:

Appendix 1: Hamilton-Reeves JM, Rebello SA, Thomas W, Slaton JW, Kurzer MS. Isoflavone-rich soy protein isolate suppresses androgen receptor expression without altering estrogen receptor- β expression or serum hormonal profiles in men at high risk of prostate cancer. J Nutr. 2007;137:1769-75.

Effect of High Omega-3 Fatty Acid Diet on Markers of Breast Cancer Risk in Postmenopausal Women

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Sex hormone mediated cancers, such as breast cancer, present a significant problem in the United States. It is important to develop safe and effective preventative strategies for these diseases. Epidemiological evidence and animal studies show that dietary fat is associated with risk of development of sex hormone mediated cancer. Specifically that a high intake of omega-6 fatty acids increases risk while omega-3 fatty acids are associated with risk reduction. Although the associations between dietary fat and sex hormone mediated cancers is unclear, it is likely due to mechanisms of endocrine balance, eicosanoid production, or immune function.

The primary objective of this investigation is to determine whether diets designed to increase plasma omega-3 fatty acid concentrations (a low fat diet, with or without omega-3 fatty acid enrichment), will favorably affect sex hormone distribution in postmenopausal women in a direction associated with reduced risk of sex hormone-mediated cancer development. The specific aims of this study are to evaluate the effects of total fat and omega-3 fatty acid intake on plasma sex hormone levels in postmenopausal women.

In order to evaluate these relationships we are conducting a well-controlled feeding study to evaluate dietary fat and fatty acid effects. The diets being tested in 8 week feeding periods include a "high risk" American diet (40% fat; HF), a low fat diet (20% fat; LF) and a low fat diet with supplemental omega-3 fatty acids (23% fat; LFn3). Endpoint measures of plasma sex hormones were obtained at baseline and 8 weeks of each dietary treatment.

Plasma estradiol (E₂), estrone (E₁), estrone sulfate (E₁-S), testosterone (T), androstenedione (AS), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) were analyzed by radio-immunoassay for 10 participants. SHBG levels were significantly increased at 8 weeks with LFn3 compared to LF ($p < 0.05$), and there was a trend for decreased DHEAS level at 8 weeks with LFn3 compared to LF ($p < 0.15$). A trend for increased E₂ was observed with HF compared to both LF and LFn3 at 8 weeks ($p < 0.15$). A trend for decreased E₁ and FSH was observed from baseline to 8 weeks with LFn3 ($p < 0.15$). No statistically significant differences were observed between treatments for A, T, E₁-S, or DHEA.

Preliminary results suggest that LFn3 alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women. LFn3 significantly increased plasma SHBG and decreased DHEAS concentrations in postmenopausal women compared to LF at 8 weeks. Within the LFn3 group, trends were observed for decreased E₁ and FSH from baseline to 8 weeks. A trend for elevated E₂ level was observed with HF relative to LF and LFn3 at 8 weeks. The full effects of the three diets on plasma sex hormone profile will be further elucidated as more subjects complete the study.